Antimicrob. Agents Chemother. doi:10.1128/AAC.01230-08

Title: Permeation of chlorhexidine from alcoholic and aqueous solutions within excised human skin

Short running title: Skin penetration of chlorhexidine

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Key words

Skin permeation, antiseptic, HPLC, Franz diffusion cell, chlorhexidine

Dear Editor,

Chlorhexidine digluconate (CHG) is widely used in the clinical setting for skin antisepsis prior to incision or insertion of medical devices for example central venous catheters (11-13), however, its permeation into skin is limited (6, 7, 9, 10, 16). In a recent study, we demonstrated the limited penetration of CHG in a skin model comprising full thickness excised human skin following application of 2% (w/v) aqueous CHG (9). The aim of this current study was to compare the penetration of chlorhexidine into skin following topical application of 2% (w/v) CHG in 70% (v/v) iso-propyl alcohol (IPA) compared to aqueous CHG.

Skin permeation studies were performed on full-thickness excised human skin as described previously (9). Many differences between animal models and human skin absorption have been shown to be permeant-specific, and due to the applied nature of this work, it was considered that human skin must be used (3, 5). Due to the limitation with availability of fresh human skin, frozen skin is most often used as a model to minimize interpersonal variability on the donor skin permeability. Human skin has benefits over animal skin and storage of human skin for a prolonged times has been shown not to have a significant effect on skin permeability (3, 5). In brief, excised human skin was exposed to aqueous and alcoholic CHG for clinically relevant time periods of 2 min and 30 min in a Franz cell diffusion model. The concentration of CHG in serial skin sections was determined by high-performance liquid chromatography.

Overall, following a 2 and 30 min exposure, skin penetration of CHG from both aqueous and alcoholic solutions was limited (Figures 1 and 2). At skin depths of ≥300

µm the concentration of CHG detected from both solutions was negligible (<0.0008 µg/ mg tissue). In addition, CHG did not completely permeate through the full thickness human skin model.

The concentration of CHG recovered within the top 100 µm skin sections was significantly less following a 2 min exposure to alcoholic CHG than that following similar exposure to aqueous CHG [mean CHG concentration (± s.e.) of 0.023 (± 0.007) µg and 0.157 (± 0.047) µg per mg tissue for CHG/IPA and CHG respectively, ($p = 0.008$)]. Following a 30 min exposure, there was no significant difference in skin penetration of CHG from alcoholic and aqueous solutions within the model ($p>0.05$).

The results from this study clearly demonstrate the limited permeation of CHG within a human skin model following application of either alcoholic or aqueous solutions. Moreover, the negligible concentrations of CHG detected at skin depths of >300 µm may indeed allow for microorganisms residing in the deeper layers, for example around hair follicles, to survive the skin antisepsis procedures recommended in the current EPIC guidelines (13).

Whilst chlorhexidine in alcoholic solution has clearly been shown to have superior antimicrobial activity compared to aqueous CHG (1, 8), their efficacy in reducing catheter colonization and infection is comparable (14). Alcohol, at a concentration of 70% (v/v) has rapid antimicrobial activity against a broad spectrum of microorganisms (2). However, it has also been shown to extract important lipid components of the stratum corneum (SC) and to cause dehydration of SC proteins, thus potentially compromising the permeation of CHG within the skin (4, 15). These results

clearly lay the foundation for further research within the field of skin antisepsis with a view to developing improved formulation strategies for use of CHG in clinical practice.

1. Adams, D., M. Quayum, T. Worthington, P. Lambert, and T. Elliott. 2005. Evaluation of a 2% chlorhexidine gluconate in 70% isopropyl alcohol skin disinfectant. J Hosp Infect **61:**287-90.

2. Adams, D. H. 2006. An evaluation of three strategies to reduce device related infections associated with hypodermic needles and peripheral vascular catheters. Aston University, Birmingham.

3. Bronaugh R. L., R. F. Stewart, and M. Simon. 1986. Methods for in vitro percutaneous absorption studies. VII: Use of excised human skin. J Pharm Sci 75: 1094-1097.

4. dos Anjos, J. L., D. de Sousa Neto, and A. Alonso. 2007. Effects of ethanol/l-menthol on the dynamics and partitioning of spin-labeled lipids in the stratum corneum. Eur J Pharm Biopharm **67:**406-12.

5. Harrison, S.M., Barry, B.W. & Dugard, P.H. 1984. Effects of freezing on human skin permeability. J Pharm Pharmacol 36: 261-262.

6. Hendley, J. O., and K. M. Ashe. 1991. Effect of Topical Antimicrobial Treatment on Aerobic Bacteria in the Stratum Corneum of Human Skin. Antimicrobial Agents and Chemotherapy **35:**627-631.

7. Hendley, J. O., and K. M. Ashe. 2003. Eradication of Resident Bacteria of Normal Human Skin by Antimicrobial Ointment. Antimicrobial Agents and Chemotherapy **47:**1988-1990.

8. Hibbard, J. S., G. K. Mulberry, and A. R. Brady. 2002. A clinical study comparing the skin antisepsis and safety of ChloraPrep, 70% isopropyl alcohol, and 2% aqueous chlorhexidine. J Infus Nurs **25:**244-9.

9. Karpanen, T. J., T. Worthington, B. R. Conway, A. C. Hilton, T. S. Elliott, and P. A. Lambert. 2008. Penetration of chlorhexidine into human skin. Antimicrob Agents Chemother **52:**3633-3636.

10. Lafforgue, C., L. Carret, F. Falson, M. E. Reverdy, and J. Freney. 1997. Percutaneous absorption of a chlorhexidine digluconate solution. International Journal of Pharmaceutics **147:**243-246.

11. McDonnell, G., and A. D. Russell. 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev **12:**147-79.

12. O'Grady, N. P., M. Alexander, E. P. Dellinger, J. L. Gerberding, S. O. Heard, D. G. Maki, H. Masur, R. D. McCormick, L. A. Mermel, M. L. Pearson, Raad, II, A. Randolph, and R. A. Weinstein. 2002. Guidelines for the prevention of intravascular catheter-related infections. Centers for Disease Control and Prevention. MMWR Recomm Rep **51:**1-29.

13. Pratt, R. J., C. M. Pellowe, J. A. Wilson, H. P. Loveday, P. J. Harper, S. R. Jones, C. McDougall, and M. H. Wilcox. 2007. epic2: National evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. J Hosp Infect **65 Suppl 1:**S1-64.

14. Valles, J., I. Fernandez, D. Alcaraz, E. Chacon, A. Cazorla, M. Canals, D. Mariscal, D. Fontanals, and A. Moron. 2008. Prospective Randomized Trial of 3 Antiseptic Solutions for Prevention of Catheter Colonization in an Intensive Care Unit for Adult Patients. Infect Control Hosp Epidemiol **29**:847-53.

15. Van der Merwe, D., and J. E. Riviere. 2005. Comparative studies on the effects of water, ethanol and water/ethanol mixtures on chemical partitioning into porcine stratum corneum and silastic membrane. Toxicol In Vitro **19:**69-77.

16. Wang, J. C., R. R. Williams, L. Wang, and J. Loder. 1990. In vitro skin permeation and bioassay of chlorhexidine phosphanilate, a new antimicrobial agent. Pharm Res **7:**995-1002.

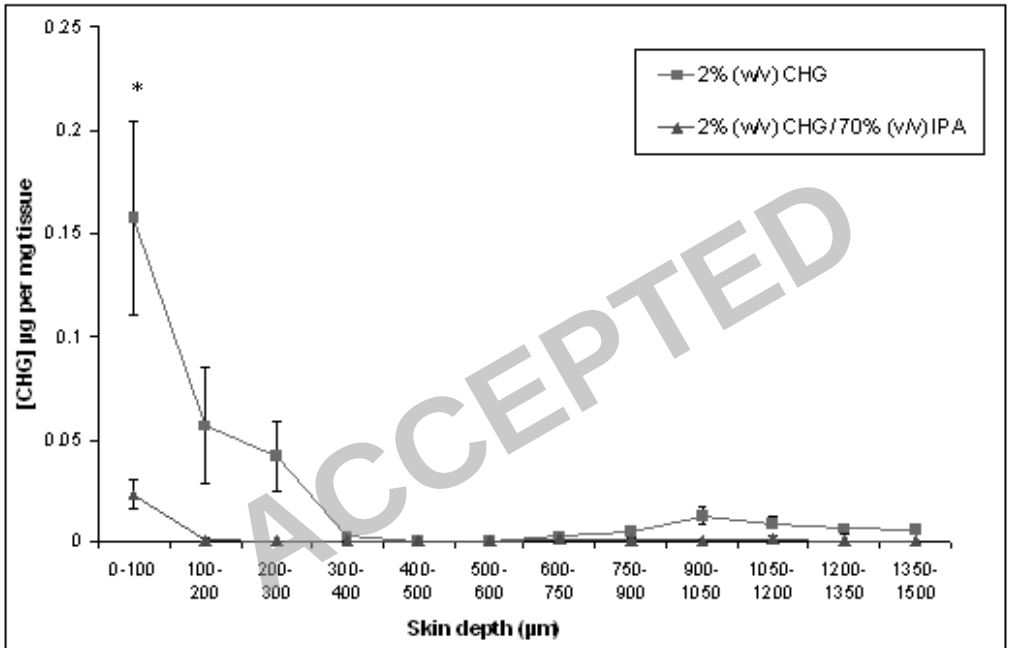

Figure 1. Penetration profile showing the location and concentration of chlorhexidine (µg/ mg tissue) in excised human skin after 2 min (n=15) exposure to 2% (w/v) chlorhexidine digluconate in 70% (v/v) isopropyl alcohol and aqueous 2% (w/v) CHG (mean ± *s.e*; *p=0.008).

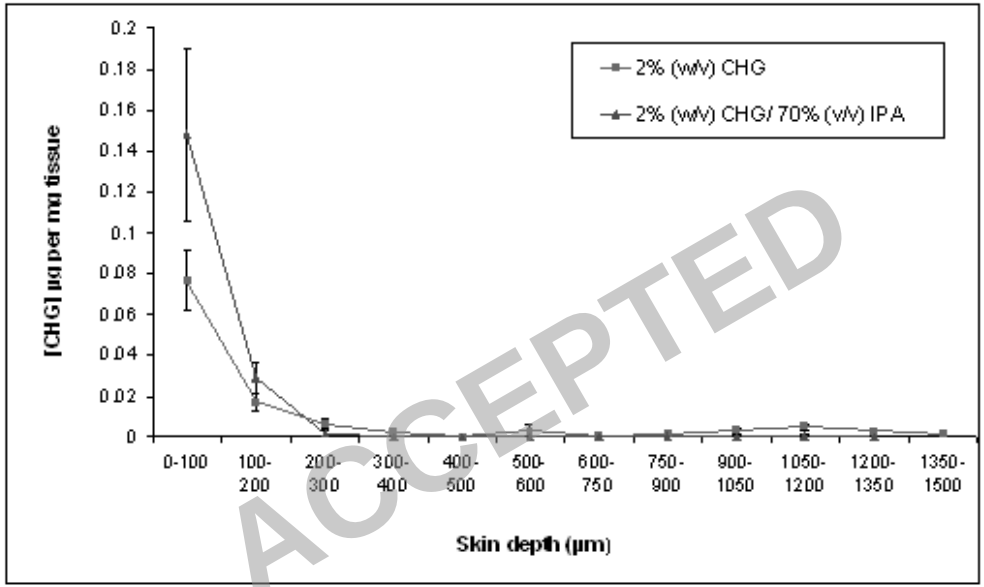

Figure 2. Penetration profile showing the location and concentration of chlorhexidine (µg/ mg tissue) in excised human skin after 30 min (n=15) exposure to 2% (w/v) chlorhexidine digluconate in 70% (v/v) isopropyl alcohol and aqueous 2% (w/v) CHG (mean ± s.e).